HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb cyano
Mobile phase: MeCN:pH 5.6 buffer 30:70
Flow rate: 1.4
Injection volume: 5
Detector: UV 225

CHROMATOGRAM
Retention time: 3.3
Internal standard: propranolol hydrochloride (7.9)
Limit of detection: 100 ng/mL

KEY WORDS
stability-indicating; skin

REFERENCE
Tenjarla,S.N.; Allen,R.; Mitchell,B. High-performance liquid chromatographic assay of terbutaline for preformulation studies, *J.Liq.Chromatogr.*, **1995**, *18*, 1603–1615.

SAMPLE
Matrix: urine
Sample preparation: Condition a Bond Elut C18 SPE cartridge with 3 volumes of MeOH and 2 volumes of water, dry under vacuum. Add 500 μL urine to the SPE cartridge, wash with 5 volumes of water, elute with 200 μL MeOH:50 mM pH 6 potassium phosphate buffer 50:50, add 50 μL 50 mM Na_3PO_4 to the eluate, pass argon through the mixture, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 300 mm long μBondapak phenyl
Mobile phase: MeCN:50 mM pH 5 phosphate buffer 6:94
Flow rate: 2.8
Injection volume: 25
Detector: F ex 280 em 310

CHROMATOGRAM
Retention time: 4.1
Internal standard: terbutaline

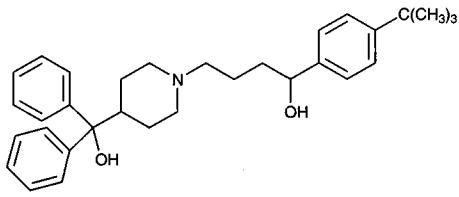
OTHER SUBSTANCES
Extracted: metaproterenol

KEY WORDS
SPE; protect from light; terbutaline is IS

REFERENCE
MacGregor,T.R.; Nastasi,L.; Farina,P.R.; Keirns,J.J. Isolation and characterization of metaproterenol-3-O-sulfate: a conjugate of metaproterenol in human urine, *Drug Metab.Dispos.*, **1983**, *11*, 568–573.

Terfenadine

Molecular formula: $C_{32}H_{41}NO_2$
Molecular weight: 471.68
CAS Registry No.: 50679-08-8
Merck Index: 9307
Lednicer No.: 4 48, 104



SAMPLE
Matrix: blood

Sample preparation: 500 μL Plasma + 50 μL 250 ng/mL IS + 5 mL MTBE:dichloromethane:n-butyl chloride 3:2:1, vortex for 1 min, centrifuge at 2500 rpm for 1 min. Froze in an acetone/dry ice bath, transfer organic layer to another tube and extract plasma layer with 5 mL MTBE:dichloromethane:n-butyl chloride 3:2:1 for a second time. Evaporate combined organic layers to dryness under a stream of nitrogen at 40°. Reconstitute in 100 μL MeCN:20 mM pH 3.5 ammonium acetate 50:50, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 2.0 5 μm TSK gel ODS-80TS (Tosho, Japan)
Mobile phase: MeCN:1% formic acid:10 mM pH 4.0 ammonium acetate 85:13:2
Flow rate: 0.2
Injection volume: 10
Detector: MS, Fisons, VG Quattro I triple quadrupole, electrospray, source 200°, cone voltage 35 V, collision energy 30 eV, parent/daughter ions 472.2/436 for terfenadine, 470.2/203.2 for IS

CHROMATOGRAM
Retention time: 2.5
Internal standard: α-[4-(1,1-dimethylethyl)phenyl]-4-(hydroxydiphenylmethyl)-1-piperidinebutanone (2.8)
Limit of quantitation: 200 pg/mL

KEY WORDS
plasma

REFERENCE
Lau,Y.Y.; Anderson,P.H.; Talaat,R. High sensitivity high performance liquid chromatography electrospray tandem mass spectrometry determination of terfenadine in human plasma, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 2669–2679.

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 20 ng/mL terfenadine-d_{10} to 1 mL heparinized plasma, vortex briefly, add 50 μL 1 M ammonia solution and 4 mL hexane, shake for 5 min on a horizontal shaker, centrifuge at 2500 rpm for 5 min, freeze the aqueous layer in a dry ice-acetone bath, evaporate the organic phase to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 10 × 2 5 μm BDS Hypersil C18
Column: 50 × 3 3 μm BDS Hypersil C18
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 54.4:32.6:13
Flow rate: 0.8
Injection volume: 30
Detector: MS, Perkin Elmer Sciex API-III, APCI interface, nebulizer 480°, nitrogen flow 1.2 L/min, interface heater 55°, multiplier 4 kV, m/z 472.7, 437

CHROMATOGRAM
Retention time: 1.10-1.15
Internal standard: terfenadine-d_{10} (1.1)
Limit of quantitation: 100 pg/mL

KEY WORDS
plasma

REFERENCE
Xu,A.; Linderholm,K.; Peng,L.; Hulse,J. Development and validation of an LC-MS-MS method for the determination of terfenadine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1675–1680.

SAMPLE
Matrix: blood, tissue

Sample preparation: Blood. Dilute 1 mL plasma with 100 μL 1 M pH 7 phosphate buffer, add 100 μL MeCN and 8 mL diethyl ether, extract. Evaporate the organic layer, dissolve the residue in 400 μL mobile phase. Inject 200 μL aliquot. Tissue. Homogenize the brain with 2 fold the weight of 1 M glycine-5 M NaOH pH 10 buffer. Extract 1500 μL brain homogenate with 22 mL n-hexane. Evaporate the organic layer and dissolve the residue in 400-500 μL mobile phase. Inject a 200 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 Intersil PH
Mobile phase: MeCN:0.018% TFA 32:68 (plasma), MeCN:0.018% TFA 37.5:62.5 (tissue)
Column temperature: 40
Flow rate: 0.7
Injection volume: 200
Detector: UV 220

CHROMATOGRAM
Limit of quantitation: 10 ng/mL (plasma), 25 ng/mL (brain)

KEY WORDS
brain; cat; mouse; pharmacokinetics; plasma; rat

REFERENCE
Kato,M.; Nishida,A.; Aga,Y.; Kita,J.; Kudo,Y.; Narita,H.; Endo,T. Pharmacokinetic and pharmacodynamic evaluation of central effect of the novel antiallergic agent betotastine besilate, *Arzneimittelforschung*, **1997**, *47*, 1116–1124.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 19.118

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: microsomal incubations

Sample preparation: 500 μL Microsomal incubation + 3 mL dichloromethane, add 100 μL buffer containing 1 M sodium carbonate, 10 mM EDTA, and 2 M NaCl, vortex for 5 min, centrifuge at 2000 g for 10 min. Evaporate the organic phase to dryness under a stream of nitrogen. Reconstitute the residue with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelco cyano
Mobile phase: MeCN:MeOH:12 mM pH 4.3 ammonium acetate buffer 30:30:40
Flow rate: 1.3
Detector: F ex 230 em 280

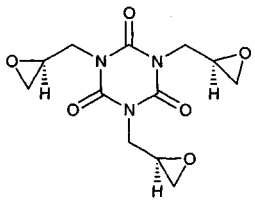
CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
Caco-2-TC7 cells; intestine; liver; pharmacokinetics

REFERENCE
Raeissi,S.D.; Guo,Z.; Dobson,G.L.; Artursson,P.; Hidalgo,I.J. Comparison of CYP3A activities in a subclone of Caco-2 cells (TC7) and human intestine, *Pharm.Res.*, **1997**, *14*, 1019–1025.

Teroxirone



Molecular formula: $C_{12}H_{15}N_2O_6$
Molecular weight: 297.27
CAS Registry No.: 59653-73-5
Lednicer No.: 4 122

SAMPLE
Matrix: blood, urine
Sample preparation: 1 mL Whole blood, plasma, or urine + 500 μL 10 mM pH 7.4 phosphate buffer + 5 mL chloroform, shake mechanically for 15 min, centrifuge at 3000 g. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 500 μL 10 mM pH 7.4 phosphate buffer, add 500 μL 5% diethyldithiocarbamate in water (freshly prepared), let stand at room temperature for 1 h, add 5 mL chloroform, shake, centrifuge. Remove 4 mL of the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50-100 μL toluene, inject an aliquot.

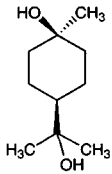
HPLC VARIABLES
Column: 10 μm PAC-CN (Whatman)
Mobile phase: Gradient. Heptane:EtOH 60:40 for 10 min, to 10:90 over 6 min.
Flow rate: 2
Detector: UV 254

CHROMATOGRAM
Retention time: 7.7
Limit of detection: 15 ng/mL

KEY WORDS
derivatization; whole blood; plasma; normal phase; human; rabbit; pharmacokinetics

REFERENCE
Ames,M.M.; Kovach,J.S.; Rubin,J. Pharmacological characterization of teroxirone, a triepoxide antitumor agent, in rats, rabbits, and humans, *Cancer Res.*, **1984**, *44*, 4151–4156.

Terpin



Molecular formula: $C_{10}H_{20}O_2$
Molecular weight: 172.27
CAS Registry No.: 80-53-5
Merck Index: 9314

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 312.8

CHROMATOGRAM

Retention time: 13.868

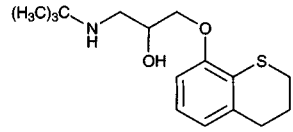
KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

Tertatolol



Molecular formula: $C_{16}H_{25}NO_2S$
Molecular weight: 295.45
CAS Registry No.: 34784-64-0
Merck Index: 9318

SAMPLE

Matrix: blood, urine
Sample preparation: Evaporate 25 μL 1 μg/mL (-)-alprenolol hydrochloride in EtOH into the bottom of a tube, add 1 mL plasma or urine, add 100 μL 1 M NaOH, vortex for 1 min, add to an Extrelut SPE cartridge, elute with two 4 mL portions of diethyl ether. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 100 μL dichloromethane, add 10 μL 0.1% S-(+)-naphthylethylisocyanate in dichloromethane, shake for 1 min, let stand

at room temperature for 12 h, add 10 μL tert-butylamine, evaporate to dryness under a stream of nitrogen, reconstitute the residue in 20 μL MeCN, vortex for 1 min, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 70 × 4.6 3 μm Ultrasphere XLODS
Mobile phase: MeCN:water 40:60
Flow rate: 2
Injection volume: 20
Detector: F ex 220 em 320

CHROMATOGRAM
Retention time: 15 (-), 17 (+)
Internal standard: (-)-alprenolol (13)
Limit of quantitation: 6 ng/mL

KEY WORDS
SPE; derivatization; chiral; plasma; pharmacokinetics

REFERENCE
Lave,T.; Efthymiopoulos,C.; Koffel,J.C.; Jung,L. Determination of tertatolol enantiomers in biological fluids by high-performance liquid chromatography, *J.Chromatogr.*, **1991**, *572*, 203–210.

SAMPLE
Matrix: saliva
Sample preparation: Condition a 100 mg 1 mL Bond-Elut C2 SPE cartridge with 1 mL MeOH, 1 mL water, and 1 mL pH 9.0 borate buffer. Centrifuge a cotton roll soaked with saliva at 1000 g for 5 min, remove the liquid supernatant. Add 1 mL supernatant to the SPE cartridge, wash with 500 μL water, wash with 500 μL MeCN, elute with two 500 μL portions of acidified MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 50 μL mobile phase, mix for 15 s, inject a 40 μL aliquot. (Acidified MeOH was 50 mL MeOH + 300 μL 96% acetic acid.)

HPLC VARIABLES
Guard column: RCSS silica guard-pack (Waters)
Column: 250 × 4.6 Chiralcel OD-H
Mobile phase: n-Hexane:EtOH:diethylamine 50:50:1
Flow rate: 1
Injection volume: 40
Detector: F ex 225 em 290 cut-off filter

CHROMATOGRAM
Internal standard: (R,S)-tertatolol

OTHER SUBSTANCES
Extracted: atenolol, pindolol

KEY WORDS
SPE; chiral; tertatolol is IS

REFERENCE
Höld,K.M.; de Boer,D.; Zuidema,J.; Maes,R.A.A. Evaluation of the Salivette as sampling device for monitoring β-adrenoceptor blocking drugs in saliva, *J.Chromatogr.B*, **1995**, *663*, 103–110.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 3.9 5 μm Nova-Pak C18
Mobile phase: MeOH:buffer 50:50 (Buffer was pH 4.0 phosphate buffer (ionic strength = 0.1) containing 4 mM N,N-dimethyloctylamine, pH readjusted to 4.00 with 85% phosphoric acid.)

Column temperature: 30
Flow rate: 1
Injection volume: 100
Detector: UV 220

CHROMATOGRAM
Retention time: k' 3.3

OTHER SUBSTANCES
Also analyzed: alprenolol, betaxolol, bopindolol, propranolol

REFERENCE
Hamoir,T.; Verlinden,Y.; Massart,D.L. Reversed-phase liquid chromatography of β-adrenergic blocking drugs in the presence of a tailing suppressor, *J.Chromatogr.Sci.*, **1994**, *32*, 14–20.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 1 mg/mL solution.

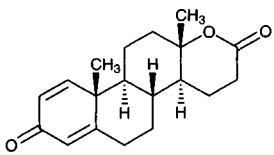
HPLC VARIABLES
Column: 250 × 4.6 10 μm Chiralcel OD
Mobile phase: Hexane:isopropanol:diethylamine 40:60:0.1
Flow rate: 0.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Retention time: k' 0.32, 1.69 (enantiomers)

KEY WORDS
chiral

REFERENCE
Ekelund,J.; van Arkens,A.; Bronnum-Hansen,K.; Fich,K.; Olsen,L.; Petersen,P.V. Chiral separations of β-blocking drug substances using chiral stationary phases, *J.Chromatogr.A*, **1995**, *708*, 253–261.

Testolactone



Molecular formula: $C_{19}H_{24}O_3$
Molecular weight: 300.40
CAS Registry No.: 968-93-4
Merck Index: 9321
Lednicer No.: 1 160

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 25 μL 10 μg/mL testosterone in MeOH + 4 mL dichloromethane, shake for 15 min, centrifuge at 2000 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 150-300 (plasma) or 300-500 (urine) μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 3 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:0.1% acetic acid 40:60
Flow rate: 1.2

Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 9.2
Internal standard: testosterone (13.6)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Pascucci,V.L.; Yeager,R.L.; Sherins,R.J.; Clark,R.V.; Gallelli,J.F.; Chatterji,D.C. Quantitation of testolactone and 4,5-dihydrotestolactone in plasma and urine using high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 79–85.

SAMPLE
Matrix: formulations
Sample preparation: Tablets. Weigh out finely-powdered tablets equivalent to about 50 mg testolactone, add 30 mL MeOH, shake vigorously for 30 min, make up to 50 mL with water, mix well, filter. Remove a 20 mL aliquot of the filtrate and mix it with 20 mL 1 mg/mL propylparaben in MeOH, make up to 100 mL with MeOH, mix, inject a 10 μL aliquot. Suspensions. Measure out an amount of suspension equivalent to about 100 mg of testolactone, add 60 mL MeOH, make up to 100 mL with water, mix well, filter (if necessary). Remove a 20 mL aliquot and mix it with 20 mL 1 mg/mL propylparaben in MeOH, make up to 100 mL with MeOH, mix, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm RP-8 (Brownlee)
Mobile phase: MeOH:water 55:45
Flow rate: 1.75
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: k' 1.89
Internal standard: propylparaben (k' 4.78)
Limit of detection: 0.1% (of testolactone)

OTHER SUBSTANCES
Simultaneous: impurities, degradation products, benzyl alcohol, 11-deoxycortisol, progesterone, testosterone

KEY WORDS
tablets; suspensions

REFERENCE
Carignan,G.; Lodge,B.A.; Skakum,W. Analysis of testolactone and its formulations by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *206*, 174–176.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

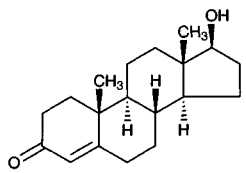
CHROMATOGRAM
Retention time: 2.9
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, testosterone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, testosterone propionate
Interfering: aspirin, caffeine, formebolone, benzyl alcohol, cortisone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

Testosterone



Molecular formula: $C_{19}H_{28}O_2$
Molecular weight: 288.43
CAS Registry No.: 58-22-0, 53608-96-1 (17-chloral hemiacetal), 58-20-8 (17β-cypionate), 315-37-7 (enanthate), 668-56-4 (nicotinate), 5704-03-0 (phenylacetate), 57-85-2 (propionate), 5874-98-6 (ketolaurate)
Merck Index: 9322
Lednicer No.: 1 172

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with MeOH and water. Add 500 μL plasma to the SPE cartridge, wash with 2 mL water, wash with 2 mL MeOH:water 20:80, elute with two 500 μL aliquots of MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 50 μL MeOH:water 20:80, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 100 × 1 5 μm Hypersil ODS
Mobile phase: MeCN:MeOH:water 25:25:50
Flow rate: 0.1
Injection volume: 20
Detector: UV (wavelength not given)

CHROMATOGRAM
Retention time: 6
Limit of detection: 2 ng/mL

OTHER SUBSTANCES
Extracted: androstenedione, 20α-hydroxy-4-pregnen-3-one, 17α-hydroxyprogesterone, norethindrone, progesterone

KEY WORDS
microbore; rat; plasma; SPE

REFERENCE
Taylor,R.B.; Kendle,K.E.; Reid,R.G.; Hung,C.T. Chromatography of progesterone and its major metabolites in rat plasma using microbore high-performance liquid chromatography columns with conventional injection and detection systems, *J.Chromatogr.*, **1987**, *385*, 383–392.

SAMPLE
Matrix: blood
Sample preparation: Extract 1 mL serum twice with 5 volumes ether by vortexing for 2 min, evaporate extracts to dryness under a stream of nitrogen at 35°, reconstitute in 100 μL MeOH.

HPLC VARIABLES
Column: 240 × 4.5 Bio-Rad ODS-5S
Mobile phase: Gradient. MeOH:MeCN:water at 20:60:20 for 3 min then to 5:85:10 over 26 min
Flow rate: 1
Injection volume: 50
Detector: UV 230

OTHER SUBSTANCES
Simultaneous: estradiol, androstenedione, progesterone

KEY WORDS
serum

REFERENCE
Yu,F.H.; Yun,Y.W.; Yuen,B.H.; Moon,Y.S. Effects of hydroxyflutamide on rats treated with a superovulatory dose of pregnant mare serum gonadotropin, *Can.J.Physiol.Pharmacol.*, **1991**, *69*, 185–190.

SAMPLE
Matrix: blood
Sample preparation: Condition a 100 mg Bond-Elut C18 SPE cartridge with 2 mL 95% EtOH and 2 mL MeCN:water 15:85. 200 μL Plasma or whole blood + 50 μL MeOH + 3 mL MeCN:water 15:85, vortex for 30 s, add to the SPE cartridge, wash with 9 mL MeCN:water 30:70, dry, elute with 200 μL 95% EtOH, inject a 10 μL aliquot of the eluate.

HPLC VARIABLES
Column: 50 × 4.6 5 μm Supelcosil LC-8DB
Mobile phase: MeOH:buffer 72.5:27.5 (Buffer was 25 mM K_2HPO_4 adjusted to pH 3 with 670 mM phosphoric acid.)
Flow rate: 1
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 3.60 (testosterone propionate)
Internal standard: testosterone propionate

OTHER SUBSTANCES
Extracted: clotrimazole, doxepin, itraconazole
Noninterfering: acetaminophen, N-acetylprocainamide, amitriptyline, aspirin, barbituric acid, brompheniramine, caffeine, carbamazepine, chloramphenicol, chlorpheniramine, clonazepam, desipramine, desmethyldoxepin, digitoxin, digoxin, disopyramide, ethosuximide, felbamate, gentamicin, ibuprofen, imipramine, lidocaine, maprotiline, mephenytoin, mephobarbital, metharbital, methsuximide, methylsuccinimide, nortriptyline, paramethadione, phenacemide, phen-

obarbital, phensuximide, phenylpropanolamine, phenytoin, primidone, procainamide, protriptyline, quinidine, theophylline, tobramycin, trimethadione, valproic acid, vancomycin

KEY WORDS
testosterone propionate is IS; plasma; SPE; whole blood

REFERENCE
Rifai,N.; Sakamoto,M.; Law,T.; Platt,O.; Mikati,M.; Armsby,C.C.; Brugnara,C. HPLC measurement, blood distribution, and pharmacokinetics of oral clotrimazole, potentially useful antisickling agent, *Clin.Chem.*, **1995**, *41*, 387–391.

SAMPLE
Matrix: blood, urine
Sample preparation: 500 μL Plasma or urine + 4 mL dichloromethane, shake for 15 min, centrifuge at 2000 g for 5 min. Remove the lower organic layer and evaporate it to dryness under a stream of air at 40°, reconstitute the residue in 150-300 (plasma) or 300-500 (urine) μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 3 30-38 μm Co:Pell ODS
Column: 250 × 4.6 5 μm Zorbax C8
Mobile phase: MeCN:0.1% acetic acid 40:60
Flow rate: 1.2
Injection volume: 100
Detector: UV 242

CHROMATOGRAM
Retention time: 13.6
Internal standard: testosterone

OTHER SUBSTANCES
Extracted: testolactone

KEY WORDS
plasma; testosterone is IS

REFERENCE
Pascucci,V.L.; Yeager,R.L.; Sherins,R.J.; Clark,R.V.; Gallelli,J.F.; Chatterji,D.C. Quantitation of testolactone and 4,5-dihydrotestolactone in plasma and urine using high-performance liquid chromatography, *J.Chromatogr.*, **1983**, *277*, 79–85.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with two 500 μL portions of MeOH and two 500 μL portions of water. 1 mL Plasma or urine + 1 mL water, add to the SPE cartridge, let stand for 2 min, wash with two 1 mL portions of water, wash with two 1 mL portions of MeOH:water 10:90, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 50 μL dry benzene (Caution! Benzene is a carcinogen!), add 5 mg potassium carbonate, add 50 μL 200 mM salicylic acid chloride in dry benzene, add 50 μL 250 mM 18-crown-6 in dry benzene, shake, heat at 70° for 1 h, cool, centrifuge, evaporate to dryness under a stream of nitrogen, reconstitute with 100 μL mobile phase, inject a 20 μL aliquot on to column A and elute to waste with mobile phase A, after 8 min elute the contents of column A on to column B with mobile phase B, elute with mobile phase B, monitor the effluent from column B. (Prepare salicylic acid chloride by stirring 27.5 g freshly distilled thionyl chloride in 30 mL dry benzene at 0° (Caution! Benzene is a carcinogen!), protect the reaction with a calcium chloride drying tube and a nitrogen atmosphere, add 25 g sodium salicylate, stir at 0° for 1 h, remove solvent by vacuum distillation, take up the residue in 50 mL dry petroleum ether, stir for 15 min, centrifuge. Remove the petroleum ether layer and evaporate it to give salicylic acid chloride.)

HPLC VARIABLES
Column: A 5 × 4 35-40 μm RP8 Perisorb (Merck); B 100 × 4.6 Spheri 5 RP8

Mobile phase: A MeOH:water 30:70; B MeOH:water 70:30 containing 2 g/L lithium perchlorate trihydrate and 2 mL/L glacial acetic acid
Flow rate: A 0.8; B 1
Injection volume: 20
Detector: E, LKB (Bromma) 2143, glassy carbon electrode +1.0 V, palladium reference electrode

CHROMATOGRAM
Retention time: 10
Limit of quantitation: 12.5 ng/mL

OTHER SUBSTANCES
Extracted: androsterone

KEY WORDS
derivatization; plasma; SPE; column-switching

REFERENCE
Wintersteiger,R.; Sepulveda,M.J. Electrochemical detection of anabolics in human plasma and urine, *Anal.Chim.Acta*, **1993**, *273*, 383–390.

SAMPLE
Matrix: formulations
Sample preparation: 1 mL Injection + 30 mL MeOH:water 90:10, shake for 15 min, centrifuge, remove the MeOH layer, repeat the extraction three times. Combine the extracts, make up to 200 mL with MeOH:water 90:10, cool to -8° for 1 h, filter an aliquot immediately. Remove an aliquot equivalent to 5 mg testosterone, add 5 mL 2 mg/mL 1,2,4,5-tetrachlorobenzene in MeOH, make up to 50 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 3.2 RP8 Express Series (Altex)
Mobile phase: MeOH:THF:water 57:11:32
Flow rate: 1
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: k' 0.60 (testosterone), k' 1.80 (testosterone acetate), k' 2.68 (testosterone propionate), k' 5.00 (testosterone benzoate), k' 6.20 (testosterone phenylpropionate), k' 12.81 (testosterone enanthate), k' 13.67 (testosterone cypionate)
Internal standard: 1,2,4,5-tetrachlorobenzene (k' 4.20)

OTHER SUBSTANCES
Simultaneous: benzyl alcohol, sesamin (from sesame oil), sesamolin (from sesame oil)

KEY WORDS
oils; injections

REFERENCE
Carignan,G.; Lodge,B.A.; Skakum,W. High-performance liquid chromatographic analysis of testosterone esters in oily solution, *J.Pharm.Sci.*, **1980**, *69*, 1214–1217.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 6.3 (testosterone), 10.8 (testosterone acetate), 25.6 (testosterone propionate)
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, trenbolone acetate, benzyl benzoate, nandrolone acetate, stanozolol, oxymetholone, nandrolone propionate, methenolone acetate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone
Interfering: nandrolone, norgestrel

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

CHROMATOGRAM
Retention time: 9.2 (testosterone), 16.2 (testosterone acetate), 24.2 (testosterone cypionate), 23.9 (testosterone enanthate), 20.0 (testosterone isobutyrate), 17.3 (testosterone propionate), 29.3 (testosterone undecanoate)

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, stanozolol
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.rling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 1 mL dichloromethane, extract, centrifuge, remove organic layer and evaporate it under vacuum, dissolve residue in 30 μL MeCN:water 50:50, centrifuge for 3 min, inject supernatant. After each run wash column with MeCN for 1 min, re-equilibrate for 1 min.

HPLC VARIABLES
Column: 50 × 4.6 3 μm Spherisorb ODS-2
Mobile phase: MeCN:water 50:50
Column temperature: 60
Flow rate: 2
Injection volume: 30
Detector: UV 200

CHROMATOGRAM
Retention time: 0.9
Limit of detection: <0.1 nmol/mL

OTHER SUBSTANCES
Simultaneous: estradiol
Interfering: estrone, androstenedione

KEY WORDS
human; placenta

REFERENCE
Taniguchi,H.; Feldmann,H.R.; Kaufmann,M.; Pyerin,W. Fast liquid chromatographic assay of androgen aromatase activity, *Anal.Biochem.*, **1989**, *181*, 167–171.

SAMPLE
Matrix: microsomal incubations
Sample preparation: Add 11β-hydroxytestosterone to microsomal incubation, extract with 5 volumes dichloromethane, evaporate the organic layer to dryness under a stream of nitrogen, reconstitute the residue in 250 μL mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Nucleosil 5 C18
Mobile phase: MeOH:water 50:50
Flow rate: 0.8
Detector: UV 254

CHROMATOGRAM
Internal standard: 11β-hydroxytestosterone

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Anderson,C.D.; Wang,J.; Kumar,G.N.; McMillan,J.M.; Walle,U.K.; Walle,T. Dexamethasone induction of taxol metabolism in the rat, *Drug Metab.Dispos.*, **1995**, *23*, 1286–1290.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 4 mL Microsomal incubation + 100 μL 10 μg/mL 11β-hydroxytestosterone + 6 mL dichloromethane, rotate for 30 min, centrifuge at 2000 g for 5 min. Remove the organic

layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 300 μL MeOH:water 25:75, vortex, inject a 100 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Hypersil C18
Column: 150 × 3.9 4 μm Novapack C18
Mobile phase: Gradient. A was MeOH:water 25:75. B was MeCN:MeOH:water 1.5:63.5:35. A:B 75:25 for 10 min, to 49.5:50.5 over 8.5 min, to 30:70 over 6.5 min, to 0:100 over 10 min, maintain at 0:100 for 15 min, return to initial conditions over 10 min, re-equilibrate for 10 min.
Flow rate: 1
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 29.5
Internal standard: 11β-hydroxytestosterone (20.8)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Ekins,S.; Murray,G.I.; Burke,M.D.; Williams,J.A.; Marchant,N.C.; Hawksworth,G.M. Quantitative differences in phase I and II metabolism between rat precision-cut liver slices and isolated hepatocytes, *Drug Metab.Dispos.*, **1995**, *23*, 1274–1279.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 1 mL Microsomal incubation + 2.5 mL cold ethyl acetate (4°), mix, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue, inject an aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm C18 (Supelco)
Mobile phase: MeCN:MeOH:water 1.3:37.8:60.9
Flow rate: 1.3
Detector: UV 240

OTHER SUBSTANCES
Extracted: metabolites, phenacetin

KEY WORDS
rat; liver

REFERENCE
Lin,J.H.; Chiba,M.; Chen,I.-W.; Vastag,K.J.; Nishime,J.A.; Dorsey,B.D.; Michelson,S.R.; McDaniel,S.L. Time- and dose-dependent pharmacokinetics of L-754,394, an HIV protease inhibitor, in rats, dogs and monkeys, *J.Pharmacol.Exp.Ther.*, **1995**, *274*, 264–269.

SAMPLE
Matrix: microsomal incubations
Sample preparation: 200 μL Microsomal incubation + 200 μL ice-cold MeOH containing 3 nmoles corticosterone, centrifuge at 1500 g for 5 min, inject a 200 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 6 × 4 10 μm μBondapak C18 Guard-Pak
Column: 250 × 4.6 5 μm Ultrasphere IP
Mobile phase: MeOH:THF:water 35:10:55 adjusted to pH 4.0 with glacial acetic acid

Flow rate: 1
Injection volume: 200
Detector: UV 245

CHROMATOGRAM
Retention time: 26.1
Internal standard: corticosterone (15.1)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; liver

REFERENCE
Sanwald,P.; Blankson,E.A.; Duléry,B.D.; Schoun,J.; Huebert,N.D.; Dow,J. Isocratic high-performance liquid chromatographic method for the separation of testosterone metabolites, *J.Chromatogr.B*, **1995**, *672*, 207–215.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Spherisorb S5-ODS2 (A), 125 × 4.5 μm LiChrospher ODS-3 (B), 250 × 4.6 10 μm Partisil 10 ODS-3 (C)
Mobile phase: MeCN:water 65:35 (A), MeCN:MeOH:water 40:30:30 (B and C)
Flow rate: 1(A), 1.5 (B), 2 (C)
Injection volume: 20 (A), 100 (B and C)
Detector: UV 270

CHROMATOGRAM
Internal standard: testosterone propionate

OTHER SUBSTANCES
Simultaneous: danazol

REFERENCE
Galia,E.; Nicolaides,E.; Hörter,D.; Löbenberg,R.; Reppas,C.; Dressman,J.B. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs, *Pharm.Res.*, **1998**, *15*, 698–705.

SAMPLE
Matrix: solutions
Sample preparation: Prepare solutions in MeCN:water 50:50, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 4 μm Sentry guard column (Waters)
Column: 150 × 3.9 4 μm mean pore diameter 60 Å Nova-Pak Phenyl
Mobile phase: THF:water 21.5:78.5
Flow rate: 1
Injection volume: 30
Detector: UV 250

CHROMATOGRAM
Retention time: 22.5

OTHER SUBSTANCES
Simultaneous: spironolactone

REFERENCE

Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE

Matrix: solutions

Sample preparation: Prepare solutions in MeCN, dilute to an appropriate concentration with mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 120 × 4.6 5 μm octadecyl Bakerbond

Mobile phase: MeCN:water 30:70 containing 16 mM β-cyclodextrin

Column temperature: 5

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 0.5

OTHER SUBSTANCES

Simultaneous: hydrocortisone, prednisone, cortisone, 17α-methyltestosterone, 17α-hydroxyprogesterone

REFERENCE

Zarzycki,P.K.; Wierzbowska,M.; Lamparczyk,H. The influence of temperature on the high performance liquid chromatographic separation of steroids using mobile phases modified with β-cyclodextrin, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 1305–1311.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 300 × 4 10 μm μPorasil

Mobile phase: Butyl chloride:water-saturated butyl chloride:THF:glacial acetic acid 55:55:3:2

Flow rate: 2-3

Detector: UV 254

CHROMATOGRAM

Retention time: 8

Internal standard: testosterone acetate

OTHER SUBSTANCES

Simultaneous: iodochlorhydroxyquin

KEY WORDS

normal phase; testosterone acetate is IS

REFERENCE

Kubiak,E.J.; Munson,J.W. Analysis of iodochlorhydroxyquin in cream formulations and bulk drugs by high-performance liquid chromatography, *J.Pharm.Sci.*, **1982**, *71*, 872–875.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 80:1.5:0.5:18
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 0.52 (testosterone), k' 8.07 (testosterone cypionate), k' 7.12 (testosterone enanthate), k' 1.95 (testosterone propionate)

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: Radial-PAK μBondapak C18
Mobile phase: MeCN:water 50:50
Flow rate: 2
Injection volume: 100
Detector: UV 254 or 214

CHROMATOGRAM
Retention time: 5.5

OTHER SUBSTANCES
Simultaneous: estrone, estriol, progesterone
Interfering: estradiol

REFERENCE
Erkoc,F.U.; Özsar,S.; Güven,B.; Kalkandelen,G.; Ugrar,E. High-performance liquid chromatographic analysis of steroid hormones, *J.Chromatogr.Sci.*, **1989**, *27*, 86–90.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher 100 RP-18
Column: 250 × 4 5 μm LiChrospher CH-18
Mobile phase: MeOH:10 mM pH 6.0 phosphate buffer 75:25 containing 2.5 mM cethexonium bromide (Rinse with 100 mL MeOH:EtOH:water 50:25:25 at the end of the day.)
Flow rate: 1
Injection volume: 10
Detector: UV 220

CHROMATOGRAM
Retention time: k' 1.3

OTHER SUBSTANCES
Extracted: glucuronides

REFERENCE
Liu,H.-F.; Leroy,P.; Nicolas,A.; Magdalou,J.; Siest,G. Evaluation of a versatile reversed-phase high-performance liquid chromatographic system using cethexonium bromide as ion-pairing reagent for the analysis of glucuronic acid conjugates, *J.Chromatogr.*, **1989**, *493*, 137–147.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH at a concentration of 100 μg/mL, inject a 5 μL aliquot.

HPLC VARIABLES
Guard column: 70 × 2.1 CO:Pell ODS
Column: 300 × 3.9 Bondex C18 (Phenomenex)
Mobile phase: MeOH:water 85:15
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 4.5 (testosterone), 6 (testosterone acetate), 8 (testosterone propionate), 13 (testosterone benzoate), 19 (testosterone enanthate), 21 (testosterone cypionate)

OTHER SUBSTANCES
Simultaneous: boldenone acetate, nandrolone propionate, boldenone benzoate, nandrolone phenylpropionate

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and mass spectral analysis of the anabolic 17-hydroxy steroid esters, *J.Chromatogr.Sci.*, **1990**, *28*, 263–268.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a 100 μg/mL solution in MeOH.

HPLC VARIABLES
Guard column: 70 × 2.1 Whatman CO:Pell ODS
Column: 300 × 3.9 Bondex C18
Mobile phase: MeOH:water 70:30
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM
Retention time: 8

OTHER SUBSTANCES
Simultaneous: methyltestosterone, nandrolone, methandrostenolone, boldenone, danazol, fluoxymesterone

REFERENCE
Noggle,F.T.,Jr.; Clark,C.R.; DeRuiter,J. Liquid chromatographic and spectral analysis of the 17-hydroxy anabolic steroids, *J.Chromatogr.Sci.*, **1990**, *28*, 162–166.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 150 × 6 5 μm Shim-pack CLC-ODS
Mobile phase: MeOH:THF:water 26:18:56
Column temperature: 48
Flow rate: 1
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 11.5

OTHER SUBSTANCES
Simultaneous: cortisone, estriol, cortisol, corticosterone, 11-deoxycortisol, androstenedione, prednisone acetate, 11-deoxycorticosterone, 17α-hydroxyprogesterone, dexamethasone acetate, estradiol, estrone, progesterone

REFERENCE
Wei,J.Q.; Wei,J.L.; Zhou,X.T. Optimization of an isocratic reversed phase liquid chromatographic system for the separation of fourteen steroids using factorial design and computer simulation, *Biomed.Chromatogr.*, **1990**, *4*, 34–38.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 0.5 mg/mL solution in MeOH, inject a 5 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 150 mM phosphoric acid and 50 mM triethylamine. B was MeCN: water 80:20 containing 150 mM phosphoric acid and 50 mM triethylamine. A:B 100:0 for 2.2 min then to 0:100 over 30 min.
Column temperature: 30
Flow rate: 2
Injection volume: 5
Detector: UV 210

CHROMATOGRAM
Retention time: 22.9 (testosterone), 30.7 (testosterone propionate)

OTHER SUBSTANCES
Simultaneous: acetaminophen, aprobarbital, butabarbital, chlordiazepoxide, chloroxylenol, chlorpromazine, clenbuterol, cortisone, danazol, doxapram, fluoxymesterone, mefenamic acid, methyltestosterone, nicotine, oxazepam, phentermine, phenylpropanolamine, progesterone, sulfamethazine, sulfanilamide, tranylcypromine, tripelennamine
Interfering: (with testosterone) diflunisal, estrone

KEY WORDS
details for purification of triethylamine in paper

REFERENCE
Hill,D.W.; Kind,A.J. The effects of type B silica and triethylamine on the retention of drugs in silica based reverse phase high performance chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 3941–3964.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine,

chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 25 μg/mL solution in mobile phase, inject an aliquot.

HPLC VARIABLES

Column: 250 × 4.6 Partisil 10 ODS-1
Mobile phase: MeOH:water 55:45
Column temperature: 40
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM

Retention time: k' 4.146

OTHER SUBSTANCES

Also analyzed: androsterone (UV 210), cortexolone (UV 240), cortisone (UV 240), estradiol (UV 280), estrone (UV 280), ethinyl estradiol (UV 280), ethisterone (UV 240), hydrocortisone (UV 240), hydroxyprogesterone (UV 240), lynestrenol (UV 210), medroxyprogesterone acetate (UV 240), medroxyprogesterone (UV 240), methandienone (UV 240), methylandrostenediol (UV 210), methylprednisolone acetate (UV 240), methylprednisolone (UV 240), methyltestosterone (UV 240), nandrolone (UV 240), norethisterone (UV 240), prednisolone acetate (UV 240), prednisolone (UV 240), prednisone (UV 240), pregnenolone (UV 210), progesterone (UV 240)

REFERENCE
Sadlej-Sosnowska,N. Structure retention relationship for steroid hormones. Functional groups as structural descriptors, *J.Liq.Chromatogr.*, **1994**, *17*, 2319–2330.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES
Column: 250 × 4 7 μm LichroCART RP-8 (Merck)
Mobile phase: MeCN:MeOH:water 32:37:31
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 16 (testosterone propionate)

OTHER SUBSTANCES
Simultaneous: fluoxymesterone, medrogestone, mestranol, norethindrone, progesterone

REFERENCE
Gau,Y.S.; Sun,S.W.; Chem,R.R.-L. Optimization of high-performance liquid chromatographic separation for progestogenic, estrogenic, and androgenic steroids using factorial design, *J.Liq.Chromatogr.*, **1995**, *18*, 2373–2382.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Nucleosil phenyl
Mobile phase: Gradient. Carbon dioxide:MeOH from 98:2 to 78:22 over 40 min
Column temperature: 50
Flow rate: 2
Detector: UV

CHROMATOGRAM
Retention time: 9.2 (testosterone), 5.6 (testosterone enanthate)

OTHER SUBSTANCES
Simultaneous: estradiol, hydrocortisone, norethisterone, hydroxyprogesterone, estriol, other steroids

KEY WORDS
SFC; 200 bar

REFERENCE
Hanson,M. Aspects of retention behaviour of steroids in packed column supercritical fluid chromatography, *Chromatographia*, **1995**, *40*, 58–68.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in n-propanol:water 80:20 or DMF:water 80:20, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm LiChrospher 100 Diol
Mobile phase: Gradient. A was hexane. B was ethyl acetate. C was 0.1% formic acid in MeCN. D was 0.1% formic acid in water. A:B:C:D 100:0:0:0 for 5 min, to 0:100:0:0 over 15 min, maintain

at 0:100:0:0 for 5 min, to 0:0:100:0 over 5 min, maintain at 0:0:100:0 for 5 min; to 0:0:0:100 over 25 min, maintain at 0:0:0:100 for 5 min.
Flow rate: 0.9
Detector: Evaporative light scattering (Sédex 55, Sédéré)

CHROMATOGRAM
Retention time: 17.71

OTHER SUBSTANCES
Simultaneous: acetylcholine, cholesterol, choline, cortisone, dextrose, estradiol, glycine, phenylalanine, sodium

REFERENCE
Treiber,L.R. Normal-phase high-performance liquid chromatography with relay gradient elution. I. Description of the method, *J.Chromatogr.A*, **1995**, *696*, 193–199.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a 100-500 μg/mL solution in mobile phase.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Hypersil C8 MOS 100A coated with phosphatidylcholine (95% pure soybean lecithin, Epikuron, Lucas Meyer & Co.) (Coat column by recycling a 1 mM solution of phosphatidylcholine in MeOH:water 80:20 for 24 h.)
Mobile phase: MeCN:35 mM pH 7.4 sodium phosphate buffer 40:60
Flow rate: 0.5–2
Injection volume: 20
Detector: UV 254

CHROMATOGRAM
Retention time: k' 3.31

OTHER SUBSTANCES
Also analyzed: amoxicillin, antipyrine, carbamazepine, chlorpheniramine, chlorpromazine, clonidine, codeine, desipramine, diphenhydramine, dipyridamole, ephedrine, flufenamic acid, haloperidol, hydroxyzine, imipramine, indomethacin, lidocaine, megestrol acetate, metoprolol, nabumetone, nadolol, phenobarbital, phenol, promazine, propranolol, pyrilamine, quinidine, ropinirole, thioridazine, tolfenamic acid, verapamil
Noninterfering: acetaminophen, aspirin, azathioprine, caffeine, carprofen, chlorambucil, cimetidine, fenoterol, flurbiprofen, ibuprofen, ketoprofen, ranitidine, salicylic acid, sulfamethoxazole, theophylline, thioguanine, tiaprofenic acid, trimethoprim, valproic acid

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Hanna,M.; de Biasi,V.; Bond,B.; Salter,C.; Hutt,A.J.; Camilleri,P. Estimation of the partitioning characteristics of drugs: A comparison of a large and diverse drug series utilizing chromatographic and electrophoretic methodology, *Anal.Chem.*, **1998**, *70*, 2092–2099.

SAMPLE
Matrix: tissue
Sample preparation: Extract 70-125 mg tissue four times with 5 mL portions of ether:chloroform 80:20. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject an aliquot.

HPLC VARIABLES
Column: 80 mm long 10 μm octadecylsilane radial compression (Radial-Pak) (Waters)
Mobile phase: Gradient. A was MeOH:water 50:50. B was MeOH. A:B form 100:0 to 70:30 over 20 min, to 0:100 over 20 min
Flow rate: 2

Detector: UV 254

CHROMATOGRAM
Retention time: 28

OTHER SUBSTANCES
Extracted: androstenedione, deoxycortisol, hydrocortisone, 17-hydroxyprogesterone
Simultaneous: estriol, estradiol, pregnenolone, progesterone, testosterone enanthate, testosterone propionate

KEY WORDS
tumor

REFERENCE
Kessler,M.J. Analysis of steroids from normal and tumor tissue by HPLC, *Clin.Chim.Acta*, **1982**, *125*, 21–30.

SAMPLE
Matrix: tissue
Sample preparation: 1 g Tissue + 10 mL Chloroform:MeOH 2:1, homogenize for 1 min (Polytron setting 5), filter, rinse tube with an additional 10 mL chloroform:MeOH, filter, combine filtrates, add 4 mL water, vortex for 1 min, centrifuge at 600 g for 10 min. Remove organic layer and dry it under air at 40°. Reconstitute with 200 μL MeOH, add to an activated Sep-Pak C18 cartridge, wash tube onto cartridge with 200 μL MeOH, elute with 5 mL each 0, 25, 50, 75, 100% MeOH, collect 5 mL fractions, inject a 100 μL aliquot of each fraction. (Elutes in 75% MeOH fraction.)

HPLC VARIABLES
Guard column: in line guard column
Column: 80 mm long 10 μm μBondapak C18 radially compressed
Mobile phase: MeCN:water 45:55
Flow rate: 3
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 5.05
Limit of quantitation: 1000 ng/g

OTHER SUBSTANCES
Simultaneous: methyltestosterone

KEY WORDS
fish; muscle; tilapia aurea; SPE

REFERENCE
Goudie,C.A. Extraction of a synthetic androgen from fish muscle and quantitation by high performance liquid chromatography, *Steroids*, **1984**, *44*, 241–252.

SAMPLE
Matrix: tissue
Sample preparation: Dry pack 60 × 8 mm glass columns with 250 mg Carbopack B (200-400 mesh) and 60 × 4 mm glass columns with 50 mg Amberlite CG-400 I (100-200 mesh). Wash Carbopack column with 5 mL MeOH, 15 mL dichloromethane:MeOH 70:30, and MeOH:water 85:15. Wash Amberlite column with 3 mL 0.5 M NaOH, 8 mL dichloromethane:MeOH 70:30, 1 mL water, and 3 mL 1 M HCl. Repeat this cycle 4 times. Finally pass through 20 mL 50 mM NaOH then 1 mL water. Keep column in water. (Process converts Amberlite to OH form.) Homogenize 1 g of tissue in 5 mL MeOH, sonicate 5 min, centrifuge at 6000 rpm for 10 min. Add another 5 mL MeOH to pellet and repeat. Combine supernatants, make up to 6.8 mL with MeOH, add 1.2 mL water. Pass through Carbopack column, wash column with 2 mL MeOH: water 85:15 then 2 mL MeOH, elute column with 8 mL dichloromethane:MeOH 70:30. Pass eluate onto Amberlite column, add 1 mL MeOH to column, collect all eluates from column,

evaporate to dryness under nitrogen at 40°, take up in 200 μL MeOH:water 50:50, add 25 μL 10 μg/mL p-chlorophenol, inject 50 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelguard LC-18
Column: 250 × 4.6 5 μm Supelco C18
Mobile phase: Gradient. MeCN:water from 40:60 to 65:35 in 30 min
Flow rate: 1.2
Injection volume: 50
Detector: UV 242

CHROMATOGRAM
Retention time: 11
Internal standard: p-chlorophenol (7)
Limit of detection: 1 ng/g

OTHER SUBSTANCES
Simultaneous: trenbolone, progesterone

KEY WORDS
muscle; liver; chicken; ox; SPE

REFERENCE
Laganà,A.; Marino,A. General and selective isolation procedure for high-performance liquid chromatographic determination of anabolic steroids in tissues, *J.Chromatogr.*, **1991**, *588*, 89–98.

SAMPLE
Matrix: urine
Sample preparation: Add 10 mL urine to a Supelclean LC-18 SPE tube at a flow rate of 2 mL/min, wash with 4 mL 25 mM sodium borate buffer, wash with 4 mL 40% MeOH, wash with 4 mL 20% acetone, elute with two 500 μL aliquots of 73% MeOH, evaporate under nitrogen at 40°, reconstitute with 1 mL mobile phase, inject a 200 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Microsorb silica
Mobile phase: Cyclohexane:ethyl acetate 40:60
Injection volume: 200
Detector: F ex 247 em 547, after post-column reaction with 30 mM $Tb(NO_3)_3$ in ethyl acetate using a 50 cm tightly coiled capillary tube to ensure mixing

CHROMATOGRAM
Retention time: 10 (testosterone acetate), 17 (testosterone)
Limit of detection: 130 pg/mL (testosterone acetate), 85 pg/mL (testosterone)

OTHER SUBSTANCES
Extracted: progesterone, bolasterone
Simultaneous: 17-methyltestosterone

KEY WORDS
SPE; normal phase; post-column reaction

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

SAMPLE
Matrix: urine
Sample preparation: Inject 200 μL directly.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Rainin C18

Mobile phase: MeCN:100 mM sodium dodecyl sulfate + 10 mM $Tb(NO_3)_3$ 20:80 (Sodium dodecyl sulfate was 99.99%.)
Column temperature: 40
Injection volume: 200
Detector: F ex 247 em 547

CHROMATOGRAM
Retention time: 8 (testosterone), 27 (testosterone acetate)
Limit of detection: 50 ng/mL (testosterone), 10 ng/mL (testosterone acetate)

OTHER SUBSTANCES
Extracted: progesterone, 17-methyltestosterone, bolasterone

KEY WORDS
micellar chromatography

REFERENCE
Amin,M.; Harrington,K.; von Wandruszka,R. Determination of steroids in urine by micellar HPLC with detection by senstized terbium fluorescence, *Anal.Chem.*, **1993**, *65*, 2346–2351.

SAMPLE
Matrix: urine
Sample preparation: 5 mL Urine + 10 μL 100 μg/mL methyltestosterone + 1 mL 200 mM pH 7.0 sodium phosphate buffer + 50 μL β-glucuronidase (E. coli K12, Boehringer Mannheim), heat at 55° for 1 h, cool to room temperature, add 1 g sodium bicarbonate:potassium carbonate 1:2, add 1 g sodium sulfate, add 5 mL n-pentane, shake for 20 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness, reconstitute the residue in 50 μL MeOH, inject a 20 μL aliquot.

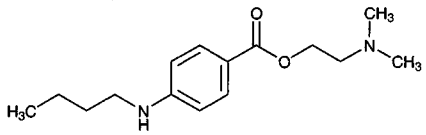
HPLC VARIABLES
Column: 250 × 4 5 μm Hypersil BDS-C18
Mobile phase: Gradient. MeCN:1 mM phosphoric acid from 25:75 to 30:70 over 10 min, to 35:65 over 6.5 min, maintain at 35:65 for 11.5 min.
Column temperature: 40
Flow rate: 1.2
Injection volume: 20
Detector: UV 240

CHROMATOGRAM
Retention time: 20
Internal standard: methyltestosterone (25)
Limit of quantitation: 20 ng/mL

OTHER SUBSTANCES
Extracted: epitestosterone

REFERENCE
Navajas,R.; Imaz,C.; Carreras,D.; García,M.; Pérez,M.; Rodríguez,C.; Rodríguez,A.F.; Cortés,R. Determination of epitestosterone and testosterone in urine by high-performance liquid chromatography, *J.Chromatogr.B*, **1995**, *673*, 159–164.

Tetracaine



Molecular formula: $C_{15}H_{24}N_2O_2$
Molecular weight: 264.37
CAS Registry No.: 94-24-6, 136-47-0 (HCl)
Merck Index: 9330
Lednicer No.: 1 110

SAMPLE
Matrix: blood
Sample preparation: Collect 10 mL blood in a bottle containing 200 µL 10% sodium metabisulfite and 200 µL 2 M NaOH, centrifuge to separate plasma. 2 mL Plasma + salicylic acid + propiophenone + 8 mL diethyl ether:dichloromethane 10:8, shake for 10 min, centrifuge at 3000 g for 5 min. Remove the organic layer and evaporate it to dryness at 50°, reconstitute the residue in 200 µL mobile phase, inject a 20 µL aliquot.

HPLC VARIABLES
Column: µBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 600 µL/L sulfuric acid, 5 g/L sodium sulfate, and 200 mg/L sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 5
Internal standard: salicylic acid (6), propiophenone (10)
Limit of detection: 20 ng/mL

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma

REFERENCE
Mazumdar,B.; Tomlinson,A.A.; Faulder,G.C. Preliminary study to assay plasma amethocaine concentrations after topical application of a new local anaesthetic cream containing amethocaine, *Br.J.Anaesth.*, **1991**, *67*, 432–436.

SAMPLE
Matrix: blood
Sample preparation: 1 mL Plasma + 100 µL 1 M NaOH + 3 mL heptane:ethyl acetate 90:10, shake for 2 min, centrifuge at 1200 g for 10 min. Remove the organic phase and add it to 50 µL 50 mM sulfuric acid, shake for 2 min, centrifuge at 1200 g for 5 min. Remove the aqueous phase and add it to 820 µg sodium acetate, inject a 40 µL aliquot. (The sodium acetate was measured out by adding 50 µL 200 mM sodium acetate in MeOH to the tube and evaporating the MeOH.)

HPLC VARIABLES
Column: 250 × 4 10 µm µBondapak C18
Mobile phase: MeCN:10 mM NaH_2PO_4 35:65, adjusted to pH 2.1
Column temperature: 30
Flow rate: 1
Injection volume: 40
Detector: UV 205

CHROMATOGRAM
Retention time: 8

Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: pramocaine

KEY WORDS
plasma; rabbit

REFERENCE
Le Guévello,P.; Le Corre,P.; Chevanne,P.; Le Verge,R. High-performance liquid chromatographic determination of bupivacaine in plasma samples for biopharmaceutical studies and application to seven other local anaesthetics, *J.Chromatogr.*, **1993**, *622*, 284–290.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Plasma + 100 μL 20 μg/mL propentofylline + 8 mL dichloromethane, shake for 20 min, centrifuge at 2500 rpm for 20 min. Remove 7 mL of the organic layer and evaporate it to dryness under nitrogen or at 60°. Dissolve residue in 200 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 6 Shimpack CLS-ODS (Shimadzu)
Mobile phase: MeCN:MeOH:0.5 mM phosphoric acid 23:20:57
Column temperature: 40
Flow rate: 1.5
Injection volume: 20
Detector: UV 290

CHROMATOGRAM
Internal standard: propentofylline

KEY WORDS
plasma; rat

REFERENCE
Lee,C.K.; Uchida,T.; Kitagawa,K.; Yagi,A.; Kim,N.-S.; Goto,S. Skin permeability of various drugs with different lipophilicity, *J.Pharm.Sci.*, **1994**, *83*, 562–565.

SAMPLE
Matrix: blood
Sample preparation: 2 mL Whole blood or plasma + 2 mL buffer + 5 mL chloroform:isopropanol: n-heptane 60:14:26, shake gently horizontally for 10 min, centrifuge at 2800 g for 10 min. Remove the lower organic layer and evaporate it to dryness under vacuum at 45°, reconstitute the residue in 100 μL mobile phase, centrifuge at 2800 g for 5 min, inject a 50 μL aliquot of the supernatant. (Buffer was saturated ammonium chloride solution 25% diluted with water, adjusted to pH 9.5 with 25% ammonia solution.)

HPLC VARIABLES
Column: 300 × 3.9 4 μm NovaPack C18
Mobile phase: MeOH:THF:buffer 65:5:30 (Buffer was 0.68 g/L (10 mM (sic)) KH_2PO_4 adjusted to pH 2.6 with concentrated orthophosphoric acid.) (At the end of each session wash the column with water for 1 h and MeOH for 1 h, re-equilibrate for 30 min.)
Column temperature: 30
Flow rate: 0.8
Injection volume: 50
Detector: UV 313

CHROMATOGRAM
Retention time: 6.16
Limit of detection: <120 ng/mL

KEY WORDS

whole blood; plasma; interferences may occur–compounds (all of which are extracted) elute in this order tenoxicam; iproniazid; methocarbamol; methotrexate; caffeine; nialamide; colchicine; cytarabine; benzoylecgonine; acetaminophen; diazoxide; dacarbazine; sulfinpyrazole; flumazenil; sulpride; morphine; atenolol; toloxatone; terbutaline; albuterol; phenobarbital; ranitidine; tiapride; phenol; chlormezanone; aspirin; metformin; ritodrine; codeine; sultopride; amisulpride; naltrexone; lisinopril; benzocaine; nizatidine; nalorphine; mephenesin; naloxone; sotalol; carteolol; procainamide; carbamazepine; bromazepam; nalbuphine; nadolol; procarbazine; dihydralazine; omeprazole; strychnine; acebutolol; glutethimide; chlorpropamide; glipizide; triazolam; prazosin; flunitrazepam; clonazepam; metoclopramide; melphalan; estazolam; tolbutamide; ephedrine; clonidine; pindolol; clobazam; minoxidil; disopyramide; nitrazepam; dextromethorphan; tofisopam; zopiclone; debrisoquine; sulindac; alprazolam; cycloguanil; lorazepam; methaqualone; ketamine; piroxicam; metoprolol; nifedipine; quinine; mephentermine; prilocaine; pentazocine; oxazepam; tiaprofenic acid; quinidine; celiprolol; ajmaline; yohimbine; lidocaine; secobarbital; viloxazine; mepivacaine; meperidine; doxylamine; labetalol; temazepam; amodiaquine; benperidol; droperidol; hydroxychloroquine; zolpidem; ketoprofen; alminoprofen; cicletanine; moclobemide; chloroquine; cocaine; timolol; nomifensine; ticlopidine; acenocoumarol; vindesine; mexiletine; dipyridamole; trazodone; pipamperone; pyrimethamine; benazepril; vincristine; metapramine; chlordiazepoxide; oxprenolol; warfarin; clorazepate; flecainide; phencyclidine; thiopental; fenfluramine; metipranolol; triprolidine; naproxen; buprenorphine; verapamil; buspirone; tianeptine; midazolam; bupivacaine; carbinoxamine; loprazolam; cetirizine; chlorpheniramine; moperone; cibenzoline; medifoxamine; astemizole; vinblastine; nicardipine; bisoprolol; diltiazem; glibornuride; reserpine; aconitine; nitrendipine; diazepam; mianserin; ramipril; haloperidol; tetracaine; alprenolol; acepromazine; glibenclamide; chlorophenacinone; doxepin; nimodipine; diphenhydramine; cyclizine; histapyrrodine; phenylbutazone; demexiptiline; clozapine; proguanil; trifluperidol; medazepam; cyamemazine; bumadizone; suriclone; propranolol; acepromazine; dothiepin; dextromoramide; fenoprofen; dextropropoxyphene; loxapine; betaxolol; propafenone; promethazine; thioproperazine; methadone; amoxapine; quinupramine; opipramol; cyproheptadine; brompheniramine; mefenidramine; protriptyline; flurbiprofen; tetrazepam; zorubicin; prazepam; alimemazine; loperamide; imipramine; desipramine; levomepromazine; hydroxyzine; niflumic acid; penbutolol; fluvoxamine; pimozide; daunorubicin; indomethacin; maprotiline; tropatenine; etodolac; fluoxetine; amitriptyline; nortriptyline; tioclomarol; diclofenac; mefloquine; trimipramine; chlorambucil; lidoflazine; ibuprofen; floctafenine; alpidem; loratadine; chlorpromazine; clomipramine; carpipramine; thioridazine; fentiazac; clemastine; mefenamic acid; fluphenazine; prochlorperazine; penfluridol; bepridil; terfenadine; trifluoperazine

REFERENCE

Tracqui,A.; Kintz,P.; Mangin,P. Systematic toxicological analysis using HPLC/DAD, *J.Forensic Sci.*, **1995**, *40*, 254–262.

SAMPLE

Matrix: blood, urine

Sample preparation: Urine. Adjust pH of urine to 5 before freezing. Adjust pH of 5 mL urine to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 1 mL MeOH, inject a 20-100 μL aliquot. Blood. Adjust pH of 4 mL plasma or whole blood to 9.5 with borax buffer, extract twice with 7 mL portions of dichloromethane. Combine the organic layers and evaporate them to dryness under a stream of nitrogen at 60°, reconstitute the residue in 0.2-2 mL MeOH, inject a 125 μL aliquot.

HPLC VARIABLES

Column: 10 μm Radial Pak C18

Mobile phase: MeCN:16.5 mM triethylamine 85:15, pH adjusted to 3 with concentrated phosphoric acid

Flow rate: 2

Injection volume: 20-125

Detector: UV 288

CHROMATOGRAM

Internal standard: tetracaine

Limit of detection: 10 ng/mL (urine), 1 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: procaine

KEY WORDS
horse; plasma; whole blood; pharmacokinetics; tetracaine is IS

REFERENCE
Stevenson,A.J.; Weber,M.P.; Todi,F.; Mendonca,M.; Fenwick,J.D.; Young,L.; Kwong,E.; Chen,F.; Beaumier,P.; Timmings,S.; Woodard,W.; Kacew,S. Determination of procaine in equine plasma and urine by high-performance liquid chromatography, *J.Anal.Toxicol.*, **1992**, *16*, 93–96.

SAMPLE
Matrix: blood, urine
Sample preparation: 2 mL Whole blood, plasma, or urine + 1 mL saturated sodium carbonate + 50 μL 10 μg/mL butacaine, add to a 3 mL Extrelut SPE cartridge, elute with 15 mL dichloromethane. Evaporate eluate to dryness under a stream of nitrogen at 40°, reconstitute in 100 μL mobile phase, inject a 40 μL aliquot.

HPLC VARIABLES
Guard column: 5 × 6 μBondapak Guard Pak
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM ammonium acetate 50:50
Flow rate: 1.5
Injection volume: 40
Detector: UV 280

CHROMATOGRAM
Retention time: 14
Internal standard: butacaine (10)
Limit of detection: 40 ng/mL

OTHER SUBSTANCES
Extracted: procaine, p-aminobenzoic acid
Also analyzed: articaine, prilocaine, o-toluidine, lidocaine, bupivacaine, etidocaine, dibucaine, caffeine, amphetamine, ephedrine, epinephrine, morphine, monoacetylmorphine, diamorphine, ethylmorphine, codeine, acetylcodeine

KEY WORDS
whole blood; plasma; SPE

REFERENCE
Rop,P.P.; Grimaldi,F.; Bresson,M.; Fornaris,M.; Viala,A. Liquid chromatographic analysis of cocaine, benzoylecgonine, local anaesthetic agents and some of their metabolites in biological fluids, *J.Liq.Chromatogr.*, **1993**, *16*, 2797–2811.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)

Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 312.8

CHROMATOGRAM
Retention time: 13.69

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: formulations
Sample preparation: 5 mL Injection + 20 mL 10 mg/mL salicylic acid in MeOH:water 50:50 + 5 mL 10 mg/mL propiophenone in MeOH:water 50:50, make up to 50 mL with MeOH:water 50:50, homogenize (if necesary), inject a 5 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeCN:MeOH:water 20:20:60 containing 0.06% sulfuric acid, 0.5% sodium sulfate, and 0.02% sodium heptanesulfonate, pH 2.6
Flow rate: 2
Injection volume: 5
Detector: UV 305

CHROMATOGRAM
Retention time: 6
Internal standard: salicylic acid (4), propiophenone (8)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
injections; saline

REFERENCE
Menon,G.N.; Norris,B.J. Simultaneous determination of tetracaine and its degradation product, p-n-butylaminobenzoic acid, by high-performance liquid chromatography, *J.Pharm.Sci.*, **1981**, *70*, 569–570.

SAMPLE
Matrix: formulations
Sample preparation: Weigh out 1.25 g ground pastilles, add 50 mL mobile phase, stir mechanically until dissolved, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:100 mM pH 5 potassium phosphate buffer 25:75, containing 5.9 g/L NaCl and 30 mM tetrabutylammonium hydrogen sulfate
Flow rate: 2
Injection volume: 50
Detector: UV 294

CHROMATOGRAM
Retention time: 5

OTHER SUBSTANCES
Simultaneous: degradation products, n-butyl p-aminobenzoic acid, p-chloroaniline, chlorhexidine

KEY WORDS
pastilles

REFERENCE
Bauer,M.; Degude,C.; Mailhe,L. Simultaneous determination of chlorhexidine, tetracaine and their degradation products by ion-pair liquid chromatography, *J.Chromatogr.*, **1984**, *315*, 457–464.

SAMPLE
Matrix: formulations
Sample preparation: Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 220 × 4.6 5 μm Brownlee silica (Applied Biosystems)
Mobile phase: MeOH:10 mM KH_2PO_4 adjusted to pH 4.0 with 10% phosphoric acid 25:75
Flow rate: 1
Injection volume: 20
Detector: UV 235

CHROMATOGRAM
Retention time: 13.3
Limit of detection: 334 ng/mL

OTHER SUBSTANCES
Simultaneous: morphine, hydromorphone

KEY WORDS
saline; injections

REFERENCE
Venkateshwaran,T.G.; Stewart,J.T. HPLC determination of morphine-hydromorphone-bupivacaine and morphine-hydromorphone-tetracaine mixtures in 0.9% sodium chloride injection, *J.Liq.Chromatogr.*, **1995**, *18*, 565–578.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher100RP-18
Column: 125 × 4 5 μm Spherisorb ODS 2
Mobile phase: MeCN:buffer 35:65 (Buffer was 20 mM sodium acetate containing 0.28% triethylamine, adjusted to pH 4.5 with acetic acid.)
Flow rate: 1.5
Detector: UV 280

CHROMATOGRAM
Retention time: k' 2.85

OTHER SUBSTANCES
Simultaneous: 4-butylaminobenzoic acid

REFERENCE
Yang,H.; Thyrion,F.C. Determination of six pharmaceuticals and their degradation products in reversed-phase high performance liquid chromatography by using amine additives, *J.Liq.Chromatogr.Rel.Technol.*, **1998**, *21*, 1347–1357.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Guard column: 45 × 4.6 5 μm Ultrasphere ODS
Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: MeCN:MeOH:water containing 2.5 mM hexanesulfonic acid 35:40:25, adjusted to pH 6.0 with 100 mM acetic acid
Flow rate: 2
Detector: UV 310

CHROMATOGRAM
Retention time: 4.5
Internal standard: procaine (2.6)
Limit of detection: 800 pg
Limit of quantitation: 5 ng

OTHER SUBSTANCES
Simultaneous: metabolites

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Asavapichayont,P.; Hu,J.; Foldvari,M. Development of an HPLC method for simultaneous analysis of tetracaine and its metabolite in dosage forms and biological fluids, with comparison to capillary electrophoresis method (Abstract 3307), *Pharm.Res.*, **1997**, *14*, S565.

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM
Retention time: 2.7

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amiodarone, amitriptyline, antazoline, atenolol, azacyclonal, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine,

mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl, protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

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REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

SAMPLE

Matrix: solutions

Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 50:1.5:0.5:48
Flow rate: 1.5
Injection volume: 10
Detector: UV 254

CHROMATOGRAM

Retention time: 14

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions

Sample preparation: Prepare a solution in EtOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 300 × 4.1 10 μm Versapack C18
Mobile phase: MeCN:250 mM pH 2.7 potassium phosphate buffer 30:70
Flow rate: 2
Injection volume: 20
Detector: UV 235

CHROMATOGRAM

Retention time: 4.9

OTHER SUBSTANCES

Simultaneous: desmethylsertraline, sertraline

REFERENCE

Wiener,H.L.; Kramer,H.K.; Reith,M.E.A. Separation and determination of sertraline and its metabolite, desmethylsertraline, in mouse cerebral cortex by reversed-phase high-performance liquid chromatography, *J.Chromatogr.*, **1990**, *527*, 467–472.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 30 × 3.2 7 μm SI 100 ODS (not commercially available)
Column: 150 × 3.2 7 μm SI 100 ODS (not commercially available)
Mobile phase: MeCN:buffer 31.2:68.8 (Buffer was 6.66 g KH_2PO_4 and 4.8 g 85% phosphoric acid in 1 L water, pH 2.3.)
Flow rate: 0.5-1
Detector: UV 222, UV309

CHROMATOGRAM

Retention time: 2.4
Internal standard: 5-(4-methylphenyl)-5-phenylhydantoin (7.3)

OTHER SUBSTANCES

Also analyzed: aspirin, caffeine, carbamazepine, chlordiazepoxide, chlorprothixene, clonazepam, diazepam, doxylamine, ethosuximide, furosemide, haloperidol, hydrochlorothiazide, methocarbamol, methotrimeprazine, nicotine, oxazepam, procaine, promazine, propafenone, propranolol, salicylamide, temazepam, thiopental, triamterene, verapamil, zolpidem, zopiclone

REFERENCE

Below,E.; Burrmann,M. Application of HPLC equipment with rapid scan detection to the identification of drugs in toxicological analysis, *J.Liq.Chromatogr.*, **1994**, *17*, 4131–4144.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4 Lichrospher 100 RP-18
Column: 125 × 4 Lichrospher 100 RP-18
Mobile phase: MeOH:buffer 75:25 (Buffer was 320 mL 20 mM K_2HPO_4 + 680 mL 20 mM KH_2PO_4, pH 7.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 235

CHROMATOGRAM

Retention time: 9.40

OTHER SUBSTANCES

Simultaneous: benzoylecgonine, cocaine

REFERENCE

Fernández,P.; Rodríguez,P.; Bermejo,A.M.; López-Rivadulla,M.; Cruz,A. Simultaneous determination of cocaine and benzoylecgonine in vitreous humor by HPLC, *J.Liq.Chromatogr.*, **1994**, *17*, 883–890.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200

mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6

Injection volume: 25
Detector: UV 229

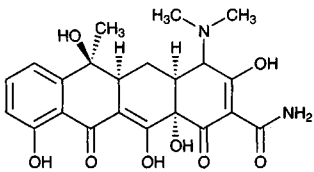
CHROMATOGRAM
Retention time: 10.81 (A), 5.44 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

Tetracycline



Molecular formula: $C_{22}H_{24}N_2O_8$
Molecular weight: 444.44
CAS Registry No.: 60-54-8, 6416-04-2 (trihydrate), 64-75-5 (HCl), 1336-20-5 (phosphate)
Merck Index: 9337
Lednicer No.: 1 212

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the